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IS 14857 (2000): Method for Determination of 2, 4-D Residues in Agricultural and Food Commodities [FAD 1: Pesticides and Pesticides Residue Analysis]



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“Knowledge is such a treasure which cannot be stolen”

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भारतीय मानक
कृषि एवं खाद्य पदार्थों में 2, 4-डी
अवशेष ज्ञात करने की विधि

Indian Standard

METHOD FOR DETERMINATION OF
2, 4-D RESIDUES IN AGRICULTURAL
AND FOOD COMMODITIES

ICS 65.100.20; 67.040; 71.040.50

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FOREWORD

This Indian Standard was adopted by the Bureau of Indian Standards, after the draft finalized by the Pesticides Residue Analysis Sectional Committee had been approved by the Food and Agricultural Division Council.

2, 4-D formulations are extensively used in agriculture as herbicide. Frequent and increased use of 2, 4-D formulations often result in harmful effects due to the toxic nature of residues. Careful assessment of residues is therefore, an important step in safeguarding human health and in the establishment of sound regulatory policy.

In the preparation of this standard, due consideration has been given to the limits of 2, 4-D residues laid down under the *Prevention of Food Adulteration Rules, 1954* and rules framed thereunder. This standard will enable the health authorities and other engaged in the field to follow uniform test procedure for the estimation of residues in - environmental samples and food commodities.

In reporting the result of a test or analysis made in accordance with this standard, if the final value, observed or calculated, is to be rounded off, it shall be done in accordance with IS 2:1960 'Rules for rounding off numerical values (*revised*)'.

Indian Standard

METHOD FOR DETERMINATION OF 2, 4-D RESIDUES IN AGRICULTURAL AND FOOD COMMODITIES

1 SCOPE

This standard prescribes the gas chromatographic method (GLC) for determination of 2, 4-D residues in environmental sample and food commodities.

2 REFERENCE

The following standard contains provisions which through reference in this text, constitutes provision of this standard. At the time of publication, the edition indicated was valid. All standards are subjected to revision and parties to agreements based on this standard are encouraged to investigate the possibility of applying the most recent edition of the standard indicated below:

<i>IS No.</i>	<i>Title</i>
11380 (Part 1) : 1985	Methods of sampling for the determination of pesticide residues : Part 1 Agricultural and food commodities

3 SAMPLING

The presentative samples for the purpose of estimating of 2, 4-D residues shall be drawn in accordance with IS 11380 (Part 1).

4 SAMPLE PREPARATION AND STORAGE

4.1 Sample Preparation

Grind 50 g of representative entire sample together with an equal weight of dry ice by using a high speed blender. Store the homogenized sample at 10°C in order to sublimate the dry ice.

4.2 Sample Storage

Sample shall be stored as such when chances of its degradation do not exist and quantity is small. Otherwise extraction shall be carried out and the extract stored. Depending upon the nature of the sample, keep the sample or the extract either in deep freezer at -10°C or in refrigerator until taken up for analysis. Ensure that the samples do not absorb or loose moisture during storage. Avoid undue long storage periods.

5 APPARATUS

5.1 Gas Chromatograph (GLC)

GLC shall be fitted with an electron capture detector (Ni63) and a printer-plotter cum integrator. The following operating parameters are suggested, which can be changed provided standardization is done:

Column	: Glass column of 2 m length, 6 mm id packed with 3 percent OV-17 on chromosorb WHP of 80-100 mesh.
Temperature:	
Column oven	: 200°C
injector	: 250°C
Detector	: 280°C
Carrier gas	: Nitrogen
Flow rate	: 30 ml/min
Miro syringe	: 2 µl

NOTE — Approximate retention time for 2, 4-D ME is 4 minutes and the minimum detectable level is 0.01 ppm under the suggested operating parameters at the signal to noise ratio 1 : 3.

5.2 High Speed Blender

5.3 Analytical Balance Capable of Weighing 0.1 mg

5.4 Mechanical Shaker

5.5 Vacuum Rotary Evaporator

5.6 Stoppered Bottles

500 ml.

5.7 Round Bottom Flasks

500 ml.

5.8 Buchner Funnel and Flask

5.9 Volumetric Pipettes

1 ml and 10 ml.

5.10 Separatory Funnels

500 ml.

5.11 Measuring Cylinder

10 ml, 250 ml.

5.12 Diazald kit (Aldrich chemical company US) or suitable apparatus for diazomethane preparation (see Fig. 1).

6 REAGENTS

6.1 Acetone

Residue grade or equivalent grade.

6.2 Chloroform

AR Grade.

6.3 Diethyl Ether

AR Grade.

6.4 Ethyl Alcohol

99.9 percent purity.

6.5 Hydrochloric Acid

2.0 N.

6.6 Sodium Bicarbonate

AR Grade.

6.7 Sodium Hydroxide

AR Grade.

6.8 N-methyl N-nitroso

20 percent w/v solution in diethylether.

6.9 Anhydrous Sodium Sulphate

AR Grade.

6.10 Filter Paper

Whatman No. 41.

6.11 Neutral Alumina

(60-100 mesh) column chromatographic grade.

6.12 Florisil

(600-100 mesh) column chromatographic grade.

6.13 2, 4-D Acid

Analytical standard of high purity.

6.14 Diazomethane

6.14.1 Diazomethane Preparation

Diazomethane is very toxic; its preparation should be carried out only in a fume cupboard (hood) provided with a powerful exhaust system. The use of a screen of safety glass is recommended.

- a) Add 50 ml of 96 percent ethanol to a solution of 10 g of potassium hydroxide in 15 ml of water. Place this solution in a 500 ml distillation flask equipped with a dropping funnel and an efficient double surface condenser. Connect the other end of condenser to a conical flask of 200 ml capacity to act as receiver, charge the conical flask with 40 ml of ether and arrange that the inlet tube of the smaller receiver dips below the surface of the ether. Cool the receiver in an ice-salt mixture. Heat the distillation flask in a water bath at 60–65°; place a solution of 43 g of N-methyl N-nitroso toluene-4-sulphonamide in about 250

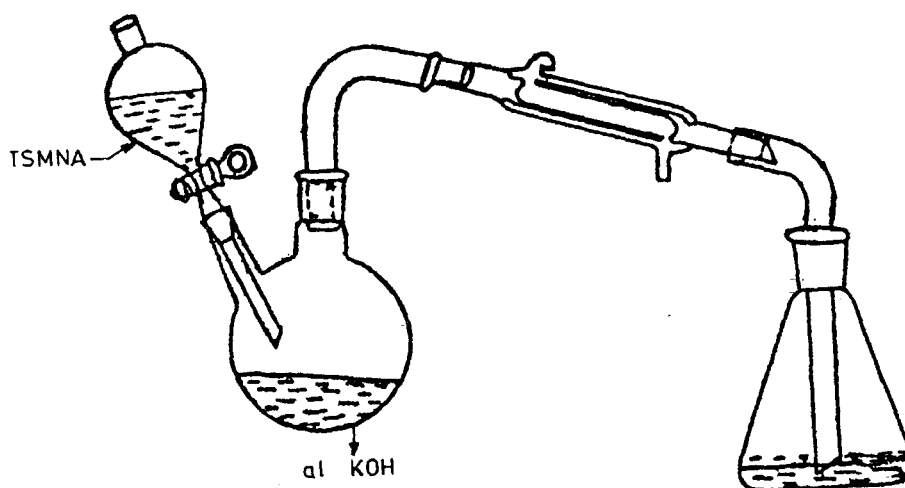


FIG. 1 DIAZO METHANE GENERATOR

ml of ether in dropping funnel and introduce it into the distillation flask over a period of 45 minutes. Adjust the rate of addition so that it is about equal to the rate of distillation. When the dropping funnel is empty, add more ether (capacity 30ml) gradually for completing ether distilling in colourless form. The combined ethereal solutions in the receivers contain 5.9-6.1 g of diazomethane.

- b) For smaller quantities of diazomethane dissolve 2.14g of N-methyl N-nitrotoluene-4 sulphonamide in 30 ml of ether, cool in ice, and add a solution of 0.4 g of potassium hydroxide in 10 ml of 96 percent ethanol. If a precipitate forms, add more ethanol until it just dissolves. After 5 minutes, distil the ethereal diazomethane solution from a water bath. The ethereal solution contains 0.32 – 0.35 g of diazomethane.

PRECAUTIONS

NOTES

- 1 The nitroso compounds from which diazomethane can be derived are all toxic and carcinogenic and should be handled with caution as they cause skin irritation (McKay, J. Amer. Chem. Soc. 1948, 70, 1974).
- 2 Any glass apparatus used with diazomethane (e.g. Diazald kit) solution should preferably be fitted with PTFE sleeves or unground joints. They may be slightly greased with mineral or silicone grease. A film of poly methylene may accumulate on the joints and prevent a tight fit. This film can be removed by a brief treatment with hot concentrated alkali and vigorous rubbing. Fire polish all glass tubes.
- 3 If peroxides are present, they may cause a violent explosion when they are concentrated by evaporation. Check the diethyl ether immediately before use with moist starch-iodide paper which turns blue if peroxides are present. See RE 12.
- 4 The reagent may also be prepared in diethyl ether (95 ml) + methanol (5 ml).
- 5 This solution is usually sufficient for between 5 and 10 ethylations.
- 6 Only potassium hydroxide pellets can be used.
- 7 Methanol may be substituted for ethanol.
- 8 Avoid inhaling the vapour from diazomethane. Prevent any skin contamination since absorption through the skin takes place from diazomethane is painful and restricted breathing. The gas has no recognizable smell.
- 9 To reduce the danger of explosion, the volume in the distillation flash should never be allowed to fall below 40 ml.
- 10 Do not expose diazomethane solutions to direct sunlight or place near a strong artificial light, because light is thought to have been responsible for some of the explosions that have been encountered with diazomethane.
- 11 Destroy any unused diazomethane solution by treatment with the acetic acid solution.

7 ESTERIFICATION OF 2, 4-D ACID

Prepare a stock solution of 10 ppm of 2, 4-D acid in ethanol. To the 1 ml of the above solution, add 3 ml of

diazomethane solution and keep the mixture in room temperature in the fuming cupboard for 10 minutes. Esterified solution is evaporated near to dryness at 30°C and taken in suitable volume of acetone.

8 EXTRACTION

8.1 Grains, straw, oilseeds, fruits, vegetables, nuts and meat.

Take 25 g of homogenized sample in 500 ml stoppered bottle, add 100 ml of acidified ether and shake in mechanical shaker for 15 minutes. Filter the organic layer and re-extract the sample with 100 ml of acidified ether, filter and combined the filtrates. Concentrate the combined filtrate to 50 ml on a rotary vacuum evaporator at room temperature.

8.2 The concentrated extract is partitioned twice with 100 ml and 50 ml of saturated sodium bicarbonate solution. The collected aqueous layer is further acidified (pH 2-3) using 2 N hydrochloric acid and partitioned twice with 150 ml of chloroform. Collect the chloroform layer each time and dry over anhydrous sodium sulphate. Evaporate the chloroform extract to near dryness and the residues are taken in small amount of methanol for column clean up.

9 CLEAN-UP

9.1 Column Clean up for Grains, Straws, Fruits, Vegetables, Nuts, Meat and Other Low Fat Products

Chromatographic column (1.8 cm id) packed with 5 g of neutral alumina (activated at 110°C) in between two layers of 2 g of anhydrous sodium sulphate using hexane is washed with 50 ml of diethyl ether. Transfer the sample solution completely into the column using 3 ml of ether. Elute the residues using 100 ml of ether and methanol (90 : 10) mixture at a flow rate of 1 ml/min. Collect the eluate, concentrate to near dryness and taken in 2 ml of ethanol for derivatization by using diazomethane as described in 9.3.

9.2 Column Clean - Up for High Fact Content Products

Chromatographic column packed with 5 g of florasil in between two layers of 2 g sodium sulphate is washed with 50 ml of solvent ether. The sample is eluted through the column as described in 9.1.

9.3 Derivatization

The samples of 9.1 and 9.2 are esterified by the addition of 3 ml of freshly prepared diazomethane. Evaporate the sample solution near to dryness and taken in suitable volume of acetone.

10 ESTIMATION

Inject 0.2 µl of reference standard solution of 2, 4-D methyl ester followed by the samples. Identify the peaks by their retention times and measure the peak heights/areas. Calculate the amount of residues of 2, 4-D as its ester from the preconstructed calibration curve.

10.0 Calculation

$$\text{2, 4-D methyl ester residues } \mu\text{g/g} = \frac{A_1 \times V \times C}{A_2 \times M} \times F$$

where

- A_1 = Peak height or area of sample injected,
 V = Total volume of the sample,
 A_2 = Peak height or area of standard injected,
 C = Concentration of standard (ppm),
 F = Recovery factor – 100/percent mean recovery, and
 M = Mass of sample in g taken for analysis.

Residues of 2, 4-D µg/g = Amount of 2, 4-D ME × 221/235.

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